nor a specific treatment to permanently restore glandular secretion, so these exciting experimental data may open new avenues for the treatment of this devastating disorder. Thus, future studies should investigate the significance of STAT3-mediated IκB-ζ-dependent increased apoptosis in human SS disease and other autoimmune pathologies in which aberrant apoptosis has been noted.

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Feeling a Little SYK after Mixing BAFF with BCR

Simona Infantino[1,*] and David M. Tarlinton[1,*]
[1]The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville 3052, Australia
*Correspondence: infantino@wehi.edu.au (S.I.), tarlinton@wehi.edu.au (D.M.T.)
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In this issue of *Immunity*, Schweighoffer et al. (2013) report that full BAFF responsiveness in B cells requires the tyrosine kinase Syk and that BAFF may co-opt components of the B cell receptor in transmitting its survival signal.

It is axiomatic to mammalian immune system function that adequate populations of recirculating B and T lymphocytes bearing unique antigen-binding receptors are maintained in peripheral lymphoid organs. The maintenance of mature B cells in this steady state requires the continuous receipt and appropriate processing of environmental cues. There are two receptors expressed by B cells that are crucial for maintaining B cell survival in the periphery; the clonally restricted B cell receptor (BCR) for antigen and the general receptor for BAFF (B cell activating factor; BLyS), a member of the TNF superfamily of receptors. Although originally considered independent, the redundancy, intersection, and crosstalk between the signaling pathways triggered by these two mediators of B cell viability have been matters of debate over many years (Mackay et al., 2010; Rickert et al., 2011). In this issue of *Immunity*, Schweighoffer et al. make a dramatic addition to this topic by providing compelling evidence that BAFF signaling impinges on components of the BCR itself and requires the kinase Syk, a key enzyme downstream of the BCR (Schweighoffer et al., 2013). Thus our understanding of BAFF-driven B cell survival appears to be incomplete and the signaling pathways emanating from the BAFFR and BCR might share common, crucial, ligand-proximal elements.

BAFFR and BCR signaling are complex and multifaceted, not all of which are required for B cell survival (Figure 1). Elegant experiments showed conclusively that the survival component of BCR signaling could be substituted completely and exclusively by a constitutively active form of the lipid-metabolizing enzyme, phosphoinositide-3-kinase (PI3K) (Srinivasan et al., 2009). Survival signaling through BAFFR, however, despite activating PI3K, depends on the noncanonical (or alternate) pathway of NF-κB (reviewed (Rickert et al., 2011)). Although the intersections of these two pathways were reviewed recently (Mackay et al., 2010; Rickert et al., 2011), it is sufficient here to note the following. First, activation of PI3K is necessary and sufficient for BCR-mediated survival and necessary but insufficient for BAFFR-mediated survival. Second, activation of PI3K and ERK downstream of BAFF is biphasic with early and late peaks, the latter requiring noncanonical NF-κB signaling (Otipoby et al., 2008; Patke et al., 2006). Third, withdrawal of BAFF or the BCR has different consequences; BCR deficiency leads to rapid loss of all B cells while loss of BAFF leaves B1 cells in the peritoneal cavity unaffected. Fourth, BCR stimulation is required to upregulate BAFFR during development, and fifth, the canonical NF-κB pathway downstream of the

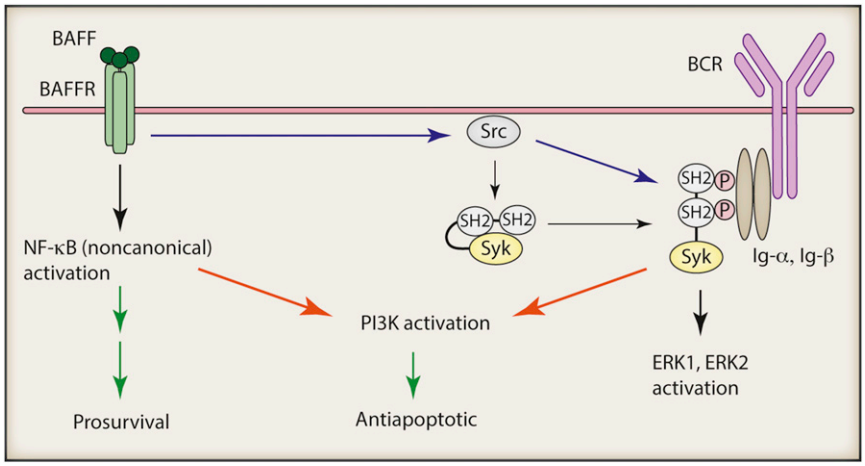

Figure 1. BAFFR Signaling Incorporating the Activation of Syk and Leading to B Cell Survival
BAFF binding to BAFFR leads to the early (min; blue) activation of Syk through the activation of Src family kinases (Src) and by binding to the phosphorylated ITAM of Ig-α. Active Syk leads to the late activation of PI3K and Akt (hr; orange), which promotes survival (green) by inhibiting proapoptotic proteins. Syk is also involved in BAFF-mediated activation of Erk1/2 (early) and the canonical NF-κB pathway (not shown). BAFF also induces Syk-independent activation of the noncanonical NF-κB pathway, leading to prosurvival gene expression (green) and late PI3K activation (orange).

BCR produces NF-κB p100, a substrate for the noncanonical pathway activated by BAFF. Whereas most of the debate on the interrelationship between BAFF and BCR has focused on the endlessly fascinating topic of the canonical and noncanonical NF-κB pathways (Mackay et al., 2010; Rickert et al., 2011), the activation of the lipid-metabolizing enzyme PI3K by BAFF remains unexplained.

Schweighoffer et al. induced the loss of Syk from mature B cells and found that it was required to transmit survival signals in follicular and marginal zone B cells but not B1 cells. The similarity with BAFF deficiency prompted examination of the involvement of Syk in BAFF responsiveness in vitro and this, unexpectedly, was found to be deficient. Continuing with unexpected outcomes, Schweighoffer et al. also discovered that BAFF induced the rapid phosphorylation of both Syk and the BCR signaling subunit, Ig-α (Figure 1), establishing a very proximal connection between BAFF and the BCR. Previous identification of PI3K as being activated by both BCR and BAFF and as mediating the BCR survival signal (Srinivasan et al., 2009) suggest that PI3K could represent the outcome of some crucial crosstalk in these two survival pathways (Figure 1). Consistent with this, Schweighoffer et al. show that Syk is required in B cells for BAFF to increase baseline phosphorylation of Akt, a known target of PI3K. The conditional deletion of PTEN or PDK1 provided additional evidence placing PI3K downstream of BAFF and Syk.

Several "simple" explanations for the requirement for Syk in BAFF signaling were excluded by Schweighoffer et al. with varying degrees of certainty. BAFFR expression and activation of the noncanonical pathway of NF-κB were shown not to be limiting in the absence of Syk for B cell survival and BAFF responsiveness. Because stimulation along the BCR and BAFFR pathways induces phosphorylation of ERK, the authors examined whether constitutively active MEK1 (caMEK1) could counteract the loss of Syk. Somewhat curiously, they found that the ectopic expression of caMEK1 enhanced the in vivo survival of Syk-deficient B cells but had no effect on BAFF responsiveness in vitro, suggesting a partial involvement in modulating B cell survival. Lastly, the requirement for Syk activity, as opposed to Syk structure, in mediating BAFF-dependent B cell survival was demonstrated by ectopically expressing Syk or Syk kinase dead (KD) in both control and Syk-deficient B cells (Schweighoffer et al., 2013).

These data support the conclusion that BAFF sustains B cell survival in vivo by mandatorily activating Syk. Is this so and if so, how, given that Syk activation requires phosphorylated ITAMs as docking sites for its two tandem SH2 domains? Schweighoffer et al. found that BAFF stimulation of B cells did indeed induce the rapid phosphorylation of Syk (Figure 1). As for the ITAM, Schweighoffer et al. provide data that Ig-α, a BCR signaling subunit, is tyrosine phosphorylated in response to BAFF (Figure 1). Although the mechanics behind BAFF-dependent Ig-α phosphorylation remain to be determined, Schweighoffer et al. show that inhibition of Src-family kinases impairs BAFF-induced Syk activation and consequently in vitro B cell survival in the presence of BAFF (Figure 1).

By showing a requirement for Syk, Schweighoffer et al. have discovered, at a minimum, exciting and unexpected new steps in BAFF signaling involving Src family kinases, phosphorylated ITAMs, and Syk, finishing in PI3K and MAPK. As the authors note, other ITAMs to which Syk can bind could contribute to BAFF signaling, but if one accepts the implication that following exposure to BAFF, phosphorylated Ig-α is a point of recruitment and activation of Syk, then this study puts a new complexion on the concept of crosstalk between BCR and BAFFR. It suggests that BAFF might activate both its own well-defined pathway and several facets of the pathways arising from the BCR, possibly activating both survival components. There remain important questions in addition to the mechanical ones already mentioned. Is there any role in this process for TACI, an alternate receptor for BAFF expressed by B cells? Is the PI3K activity induced by Syk by BAFF sufficient for survival in the absence of a BCR signal or are there different, early and late, pools of PI3K activated by these two receptors that combined mediate survival? Does the biphasic activation of PI3K by BAFF reflect Syk acting early and noncanonical NF-κB late (Figure 1)? Although unraveling these two apparently intimately linked pathways is a technically challenging proposal, it remains crucial in determining whether BCR tonic signaling is independent of, or commandeered by, BAFF.

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Basophil-Macrophage Dialog in Allergic Inflammation

Yu Hui Kang[1] and Subhra K. Biswas[1,*]
[1]Singapore Immunology Network (SIgN), Agency for Science, Technology & Research (A*STAR), #04-01 Immunos, 8A Biomedical Grove, Singapore 138648, Singapore
*Correspondence: subhra_biswas@immunol.a-star.edu.sg
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Monocyte-macrophage differentiation under pathological conditions is poorly understood. In the present issue of *Immunity*, Egawa et al. (2013) report how basophils drive the differentiation of inflammatory monocytes into M2 macrophages, thereby regulating allergic skin inflammation.

Monocytes and macrophages can perform a diverse range of functions under homeostatic and pathogenic conditions. This is facilitated by the existence of distinct subsets with specific functions and the ability to switch between different functional phenotypes in response to microenvironmental cues. Two main monocyte subsets have been recognized, namely the Ly6C^{hi}CCR2^{+} and Ly6C^{lo} CX3CR1^{+} monocytes in mice and the corresponding CD14^{+}CD16^{-} and CD16^{+} CD14^{dim} monocytes in humans (Geissmann et al., 2010). The Ly6C^{hi}CCR2^{+} "inflammatory" monocytes infiltrate inflamed tissue, produce inflammatory cytokines, and are involved in host response to infection. The Ly6C^{lo}CX3CR1^{+} monocytes "patrol" blood vessels under steady state, extravasate during tissue injury, and promote tissue repair and healing. Similarly in macrophages, at least two main types of functional activation states exist: the M1 (or classically activated) and the M2 (or alternatively activated) macrophages. Microbial stimuli and T helper 1 (Th1) cell-related cytokines (such as IFN-γ) trigger the M1 phenotype, which promotes inflammation, Th1 cell responses, and microbicidal-tumoricidal functions. Th2 cell-related cytokines (such as interleukin-4 [IL-4], IL-13, and IL-10) trigger the M2 state, which dampens excessive inflammation and promotes Th2 cell responses, tissue remodeling, protumor functions, and clearance of parasites (Biswas and Mantovani, 2010). However, M1 and M2 represent two extremes of a spectrum of macrophage functional states that may exist in vivo.

A key question arising from the above context is the relationship between the monocyte subsets and the macrophage activation states. In addition, the in vivo microenvironmental cues and interacting cells that induce monocyte-macrophage differentiation to distinct functional phenotypes, especially under pathological settings, is poorly understood. Addressing these areas, Egawa et al. (2013) in the present issue of *Immunity* investigate monocyte-macrophage differentiation in immunoglobulin E (IgE)-mediated skin allergic inflammation (IgE-CAI), uncovering a crucial role for basophils therein (Figure 1).

In mice sensitized with allergen-specific IgE, IgE-CAI was characterized by late-phase ear swelling after intradermal allergen challenge and was dependent on basophils. The skin lesions showed increased infiltration of Ly6C^{+}CCR2^{+} monocyte-macrophages and CCR2^{+} basophils and expression of CCR2 ligands CCL8 and CCL12. Based on these observations, a key role for CCR2 in the recruitment of these cells and the development of IgE-CAI was suggested. Contrary to this expectation, *Ccr2*$^{-/-}$ mice showed exacerbated IgE-CAI with abolished monocyte-macrophage infiltration in the skin lesion but enhanced infiltration of basophils (as well as neutrophils), suggesting CCR2 to be dispensable for basophil recruitment. Further investigation profiling monocyte-macrophages from IgE-CAI skin lesions of wild-type mice revealed that the majority of these cells expressed the inhibitory molecule and M2 marker, Programmed Death 1 ligand 2 (PD-L2), together with other M2 genes like *Arg1*, *Chi3l3*, and *Fizz1*, indicating an M2 phenotype (Figure 1). Experiments via adoptive transfer of CD115^{+} bone marrow monocytes or inflammatory monocytes confirmed that inflammatory monocytes infiltrate skin lesions and differentiate into M2 macrophages, which in turn dampened the allergic inflammation, as demonstrated by their ability to attenuate the exacerbated IgE-CAI in *Ccr2*$^{-/-}$ animals.

Several scenarios have emerged on the relationship between monocyte subsets